

Claims:

1. A reagent comprising:

- 5 i) a polybasic compound comprising a peptide, wherein the peptide comprises at least four arginine residues; and
- ii) a radiolabel-binding moiety covalently linked to the polybasic compound;

wherein the reagent is capable of accumulating at sites of pathology in the body.

10 2. The reagent of claim 1, wherein the peptide has from about 5 to about 100 amino acids.

15 3. The reagent of claim 1 or claim 2, wherein the reagent is capable of accumulating at sites of inflammation or infection *in vivo*.

20 4. The reagent of any of claims 1 to 3, wherein the peptide comprises an amino acid sequence corresponding to a sequence of about 5 to 70, preferably about 50, 40, 30, 20, 15, 14, 13, 12, 11, 10, or 9 contiguous amino acids of human Platelet Factor 4, or having at least 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, or 99% sequence identity to said sequence.

25 5. The reagent of claim 4, wherein said sequence of contiguous amino acids is from the C-terminus of human Platelet Factor 4 (PF4).

6. The reagent of claim 4 or claim 5, wherein the at least four arginine residues of the polybasic compound represent a substitution of corresponding lysine residues in the amino acid sequence of human Platelet Factor 4, or represent an addition to the amino acid sequence corresponding to said sequence of human Platelet Factor 4.

7. The reagent of any of claims 1 to 6, wherein the polybasic compound comprises from 4 to 9, preferably five, six, seven, eight and most preferably nine arginine residues.

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8. The reagent of any of the preceding claims, wherein the radiolabel-binding moiety is selected from the group consisting of:

I.

Cp(aa)Cp

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wherein Cp is a cysteine having a protected or unprotected thiol group and (aa) is an amino acid; or

II.

a radiolabel-binding moiety comprising a single thiol moiety, wherein the single thiol moiety has a formula:



wherein A is H, HOOC, H₂NOC, (peptide)-NHOC, (peptide)-OOC or R⁴;

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B is H, SH, -NHR³, -N(R³)-(peptide), or R⁴;

X is H, SH, -NHR³, -N(R³)-(peptide) or R⁴;

Z is H or R⁴;

R¹, R², R³ and R⁴ are independently H or lower straight or branched chain or cyclic alkyl;

25

n is 0, 1 or 2;

and where B is -NHR³ or -N(R³)-(peptide), X is SH, and n is 1 or 2;

where X is -NHR³ or -N(R³)-(peptide), B is SH, and n is 1 or 2;

where B is H or R⁴, A is HOOC, H₂NOC, (peptide)-NHOC, or (peptide)-OOC, X is SH, and n is 0 or 1;

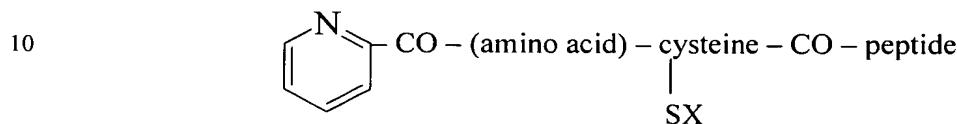
where A is H or R⁴, then where B is SH, X is -NHR³ or -N(R³)-(peptide) and where X is SH, B is -NHR³ or -N(R³)-(peptide);

where X is H or R⁴, A is HOOC, H₂NOC, (peptide)-NHOC, or (peptide)-OOC and B is SH;

5 where Z is methyl, X is methyl, A is HOOC, H₂NOC, (peptide)-NHOC, or (peptide)-OOC, B is SH and n is 0;

and wherein the thiol moiety is in the reduced form;

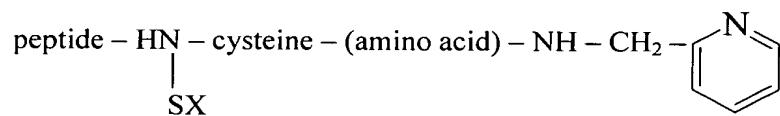
III.



wherein X = H or a protecting group;
(amino acid) = any amino acid;

15 or

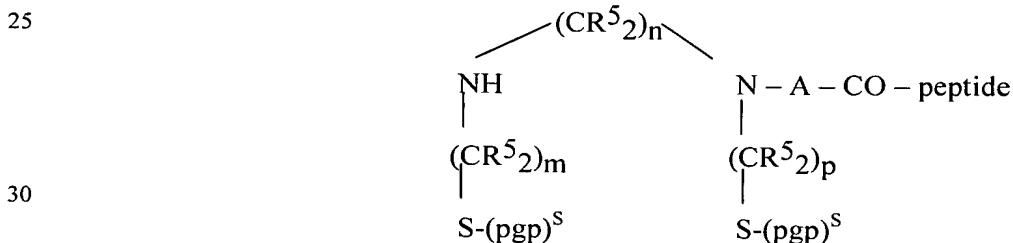
IV.



20 wherein X = H or a protecting group;
(amino acid) = any amino acid;

or

V.

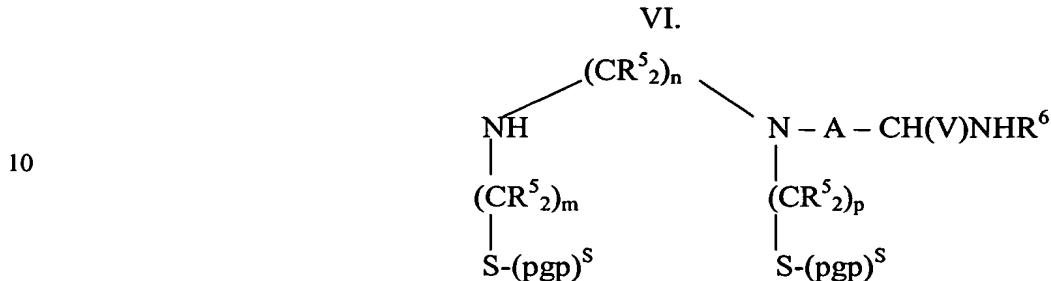


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wherein each R⁵ is independently H, lower alkyl, phenyl, or phenyl substituted with lower alkyl or lower alkoxy;

each $(pgp)^S$ is independently a thiol protecting group or H;
 m, n and p are independently 2 or 3;
 A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations
 or substituted derivatives thereof;

5 or



10 15 wherein each R^5 is independently H, lower alkyl, phenyl, or phenyl
 substituted with lower alkyl or lower alkoxy;
 each $(pgp)^S$ is independently a thiol protecting group or H;
 m, n and p are independently 1, 2 or 3;
 A = linear or cyclic lower alkyl, aryl, heterocyclyl, or
 20 combinations or substituted derivatives thereof;
 V = H or $-\text{CO-peptide}$;
 R^6 = H or peptide;

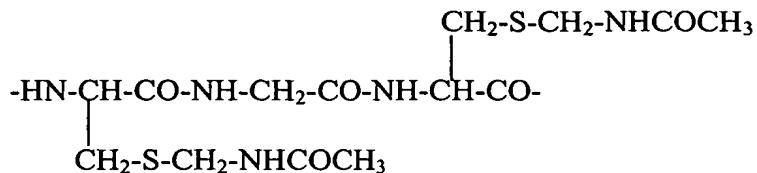
and wherein when V = H, R^6 = peptide and when R^6 = H, V = $-\text{CO-peptide}$.

25 9. The reagent of claim 8, wherein the radiolabel-binding moiety is Cp(aa)Cp and
 Cp is a protected cysteine having a protecting group of formula:



30 wherein R is a lower alkyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, phenyl, or phenyl
 substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower
 alkoxycarbonyl.

10. The reagent of claim 9, wherein the radiolabel-binding moiety has the formula:



11. The reagent of any of the preceding claims, wherein the polybasic compound and the radiolabel-binding moiety are covalently linked through from about one to about twenty amino acids.
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12. The reagent of claim 11, wherein the amino acid covalently linking the polybasic compound and the radiolabel-binding moiety is one or more glycines.
- 15 13. The reagent of any of the preceding claims, wherein the reagent comprises the amino acid sequence

KKKKKCGCGGPLYKKIICKLLES (SEQ ID No. 2),

except that at least four, preferably five, six, seven, eight and most preferably nine of the lysine residues of said peptide are substituted by arginine residues.

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14. The reagent of any of the preceding claims, wherein the polybasic compound and the radiolabel-binding moiety covalently linked thereto together form a peptide having an amino acid sequence selected from the group consisting of:

Acetyl-RRRRRCGCGGPLYRRIIRRLLES (SEQ ID No. 3);

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Acetyl-RRRRRCGCGGPLYKKIICKLLES (SEQ ID No. 4); and

Acetyl-KKKKKCGCGGPLYRRIIRRLLES(SEQ ID No. 5).

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15. The reagent of any of the preceding claims, wherein the polybasic compound and the radiolabel-binding moiety covalently linked thereto together form a peptide having the amino acid sequence:

Acetyl-RRRRRCGCGGPLYRRIIRRLLES (SEQ ID No. 3).

16. A multimeric reagent comprising

- 5 i) at least two polybasic compounds as defined in any of the preceding claims which may be the same or different;
- ii) at least one radiolabel-binding moiety as defined in any of the preceding claims covalently linked to at least one of the polybasic compounds; and
- 10 iii) a polyvalent linker moiety covalently linked to the polybasic compounds, the radiolabel-binding moieties or both;

wherein the molecular weight of the multimeric polyvalent reagent is less than about 20,000 Da.

17. The multimeric reagent of claim 16, wherein the polyvalent linking moiety is

15 comprised of at least 2 linker functional groups capable of covalently bonding to the polybasic compounds or the radiolabel-binding moieties, preferably wherein at least 2 of the linker functional groups are identical; optionally wherein the linker functional groups are primary or secondary amines, hydroxyl groups, carboxylic acid groups or thiol-reactive groups, the thiol-reactive groups being 20 selected from maleimido groups and chloroacetyl, bromoacetyl and iodoacetyl groups.

18. The multimeric reagent of claim 16 or claim 17, wherein the polyvalent linker is selected from the group consisting of:

- 25 *bis*-succinimidylmethylether;
- 4-(2,2-dimethylacetyl)benzoic acid;
- tris*(succinimidylethyl)amine;
- bis*-succinimidohexane;
- 4-(O-H₂CO-Gly-Gly-Cys.amide)acetophenone;

tris(acetamidoethyl)amine;

bis(acetamidomethyl)amine;

bis(acetamidoethyl)amine;

α,ϵ -*bis*(acetyl)lysine;

lysine; and

1,8-*bis*-acetamido-3,6-dioxa-octane;

or a derivative of any of the above-listed polyvalent linkers.

19. A complex formed by either, (a) reacting a reagent as defined in any of claims 1
10 to 18 with technetium-99m in the presence of a reducing agent, preferably a
reducing agent selected from the group consisting of a dithionite ion, a stannous
ion, and a ferrous ion, or (b) labeling the reagent as defined in any of claims 1 to
18 with technetium-99m by ligand exchange of a prereduced technetium-99m
complex.

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20. A composition comprising
- (a) the reagent as defined in any of claims 1 to 18
 - (b) a polysulfated glycan having a molecular weight of at least about 1000
Da;

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wherein the composition is capable of accumulating at sites of pathology in the
mammalian body.

21. The composition of claim 20, wherein the polysulfated glycan is dextran sulfate,
25 chondroitin sulfate, dermatan sulfate or dermatan disulfate, or any derivative or
mixture thereof, preferably wherein the polysulfated glycan is dermatan sulfate
or dermatan disulfate.

22. The composition of claim 20 or claim 21, wherein the (w/w) ratio of the polybasic compound to the polysulfated glycan is from 0.1:1 to 20:1, preferably from 0.2:1 to 10:1, more preferably from 0.5:1 to 5:1 or 1:1 to 2:1, and is most preferably about 1.45:1 or 1.5:1.

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23. The composition of any of claims 20 to 22, wherein the composition is capable of accumulating at sites of inflammation or infection *in vivo*.

24. The composition of any of claims 20 to 23, wherein the composition is capable of achieving an image contrast ratio $I_{max}:C$ between muscle tissue infected by *E. coli* and uninfected muscle tissue in the rabbit injection model of more than 25, preferably more than 40, and most preferably more than 60, and / or wherein the composition is capable of achieving an image contrast ratio $I_{max}:B$ between muscle tissue infected by *E. coli* and terminal blood in the rabbit injection model

10 of more than 3, preferably more than 4, 5, 6, 7, or 8 and most preferably more than 9;

15 when the reagent of the composition is labeled with Tc-99m and administered together with the polysulfated glycan.

20 25. The composition of any of claims 20 to 24, wherein the reagent is a peptide having the sequence

Acetyl-RRRRRCGCGGPLYRRIIRRLLES (SEQ ID No. 3),

and wherein the polysulfated glycan is dermatan sulfate.

25 26. A scintigraphic imaging agent comprising

- (a) the composition of any of claims 20 to 25; and
- (b) a radioisotope,

wherein the radioisotope is complexed to the reagent within the composition via its radiolabel-binding moiety.

27. The scintigraphic imaging agent of claim 26, wherein the radioisotope is selected from the group consisting of technetium-99m, fluor-18, gallium-67, gallium-68, indium-111, iodine-123, iodine-125, ytterbium-169, or rhenium-186.

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28. The scintigraphic imaging agent of claim 26 or claim 27, wherein the radioisotope is technetium-99m.

29. The scintigraphic imaging agent of any of claims 26 to 28, wherein the imaging agent achieves an image contrast ratio $I_{max}:C$ between muscle tissue infected by *Escherichia coli* and uninfected muscle tissue in the rabbit injection model of more than 25, preferably more than 40, and most preferably more than 60, and / or wherein the imaging agent achieves an image contrast ratio $I_{max}:B$ between muscle tissue infected by *E. coli* and terminal blood in the rabbit injection model of more than 3, preferably more than 4, 5, 6, 7, or 8 and most preferably more than 9.

30. A pharmaceutical composition comprising the reagent as defined in any of claims 1 to 18, the complex as defined in claim 19, the composition as defined in any of claims 20 to 25, or the scintigraphic imaging agent as defined in any of claims 26 to 29, further comprising a pharmaceutically acceptable carrier.

31. The reagent of any of claims 1 to 18, the complex of claim 19, the composition of any of claims 20 to 25, the scintigraphic imaging agent of any of claims 26 to 29, or the pharmaceutical composition of claim 30 for use for imaging a site of pathology within a mammalian body.

32. The reagent, the complex, the composition, the scintigraphic imaging agent, or the pharmaceutical composition of claim 31, wherein the site to be imaged is a site of inflammation or infection.

33. Use of the reagent of any of claims 1 to 18, the complex of claim 19, the composition of any of claims 20 to 25, the scintigraphic imaging agent of any of claims 26 to 29, or the pharmaceutical composition of claim 30 in the manufacture of a diagnostic pharmaceutical for imaging a site of pathology within a mammalian body.

5 34. The use according to claim 33, wherein the site to be imaged is a site of inflammation or infection.

10 35. A kit for preparing a radiopharmaceutical preparation, said kit comprising

(a) a first sealed vial containing

(i) a predetermined quantity of a reagent as defined in any of claims 1 to 18; and

15 (ii) a sufficient amount of a reducing agent to label the reagent with a radioisotope; and

(b) a second sealed vial containing a predetermined quantity of a polysulfated glycan as defined in any of claims 20 to 23.

20 36. The kit of claim 35, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion, a ferrous ion.

37. The kit of claim 35 or claim 36, wherein the reagent has the formula:

Acetyl-RRRRRCGCGGPLYRRIIRRLLES (SEQ ID No. 3); and

25 wherein the polysulfated glycan is dermatan sulfate.

38. The kit of any of claims 35 to 37, wherein the radioisotope is technetium-99m.

39. A process for preparing a reagent as defined in any of claims 1 to 18 by *in vitro* chemical synthesis.

40. The process of claim 39, wherein the reagent is prepared by solid phase peptide synthesis.

5 41. The process of claim 39 or claim 40, wherein the radiolabel-binding moiety is covalently linked to the peptide during solid phase peptide synthesis.

10 42. A method of imaging a site of pathology within a mammalian body comprising the steps of:

a) administering an effective diagnostic amount of a scintigraphic imaging agent as defined in any of the claims 26 to 29 or a pharmaceutical composition as defined in claim 30; and

15 b) detecting a radioactive signal from the radiolabel localized at said site.

43. The method according to claim 42, wherein the radiolabel is localized at a site of inflammation or infection.

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44. The method according to claim 42 or claim 43, wherein the reagent is a peptide selected from the group consisting of:

Acetyl-RRRRRCGCGGPLYRRIIRRLLES (SEQ ID No. 3);

Acetyl-RRRRRCGCGGPLYKKIIRRLLES (SEQ ID No. 4); and

25 Acetyl-KKKKKCGCGGPLYRRIIRRLLES (SEQ ID No. 5).

45. The method according to any of claims 42 to 44, wherein the polysulfated glycan is dermatan sulfate.

46. The method according to any of claims 42 to 45, wherein the radioisotope is technetium-99m.

5 47. A method of imaging a site of inflammation or infection within a mammalian body comprising the steps of:

(a) mixing whole blood and from about 1 microgram to 100 milligrams of the scintigraphic imaging agent of any of claims 26 to 29 or the pharmaceutical composition of claim 30;

10 (b) administering said mixture to a mammal; and

(c) detecting a radioactive signal from the radioisotope localized at said site.

48. The method of claim 47, wherein the radioisotope is technetium-99m.